STUDY OF EQUILIBRIA IN ALUMINUM(III) - L-GLUTAMIC ACID OR -L-SERINE SOLUTIONS

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ABSTRACT

Hydrolytic and complex formation equilibria in L-glutamic acid (H_2Glu) and L-serine (HSer) + Al^{III} systems have been studied by glass electrode potentiometric titrations in 0.1 mol/dm^3 LiCl medium, at 298 K. In the concentration range $0.6 < [Al^{III}] < 5.0$ mmol/dm^3, aluminum(III)-ion hydrolyzes between pH 3.5 and 4.5. The model which gives the best fit to the experimental data includes the species: $Al(OH)^{2+}$, $Al(OH)_2^{+}$, $Al_2(OH)_2^{4+}$, $Al_3(OH)_4^{5+}$ and $Al_{13}(OH)_{32}^{7+}$. In the L-glutamic acid + Al^{III} system, in the concentration ranges $1.0 \leq [Ai^{III}] < 5.0$, $1.5 < [Glu^{2-}] \leq 12.5$ mmol/dm^3 and pH rbetween 3.0 and 5.5, besides pure hydrolytic species formation of the following complexes (stability constants given in parenthesis) has been evidenced: $Al(HGlu)^{2+}$ ($\log \beta_{1,1,1} = 12.02 \pm 0.04$), $Al(Glu)^{+}$ ($\log \beta_{1\,0,1} = 7.86 \pm 0.01$), $AlH_{-2}Glu^{-}$ ($\log \beta_{1,-2,1} = -2.30 \pm 0.08$) and $AlH_{-3}Glu^{2-}$ ($\log \beta_{1,-3,1} = -8.44 \pm 0.10$). In the L-serine + Al^{III} system, in the concentration ranges $1.0 \leq [Al^{III}] < 10.0$, $5.0 \leq [Ser] < 25.0$ mmol/dm^3 and pH between 2.7 and 4.8, the following complexes are formed: $Al(HSer)^{3+}$ ($\log \beta_{1,1,1} = 11.16 \pm 0.03$), $Al(Ser)^{2+}$ ($\log \beta_{1,0,1} = 5.71 \pm 0.02$), $Al_2H_{-1}Ser^{4+}$ ($\log \beta_{2,-1,1} = 4.65 \pm 0.03$), $AlH_{-2}Ser$ ($\log \beta_{1,-2,1} = -2.51 \pm 0.04$) and $AlH_{-3}Ser^{-}$ ($\log \beta_{1,-3,1} = -7.5 \pm 0.1$) as well as several pure hydrolytic complexes. The mechanism of the formation of the complexes is discussed. Their role in absorption, transport, excretion and cellular uptake of aluminium is briefly discussed.

INTRODUCTION

In a previous paper [1] we have reported the solution equilibria in glycine and L-alanine + Ai^{III} systems. We found that Al^{III} does not form binary complexes with either Gly or L-Ala up to the concentration ratio of amino acid to aluminum 10:1 and pH up to 4.5. In the present paper we continue to study the complex formation of Ai^{III} with protein amino acids which, for the difference from the former two, contain a reactive, potentially chelating, polar side chain: L-glutamic acid (H_2Glu) and L-serine (HSer).

These amino acids, besides their function as building blocks for proteins, are engaged in many other fundamental processes in organisms, as precursors to numerous important bio-molecules, neurotransmitters, transporters, etc. [2]. The data accumulated over the past two decades suggest that aluminium is a toxic element that plays a significant role in the pathogenesis of many health disorders [3,4].

Glutamic acid and serine are the most metabolically active between the protein amino acids, and potential chelators for Al, too. By binding to glutamate or serinate ions trace amounts of aluminium may possibly disrupt many metabolic processes in organisms. Accordingly, the knowledge of speciation in the systems L-glutamic acid + Al^{3+} or L-serine + Al^{3+} can elucidate the metabolic fate of aluminum in body tissues. Hence, the primary aim of this work is to provide reliable data concerning identity and stability of the species formed in solutions of aluminium ion and L-glutamic acid or L-serine, *in vitro*, so that they could be used in modeling studies of metabolic transformations of aluminium *in vivo*. These data may also be important in computer modeling of solution equilibria involving aluminium ions in plasma [5]. Since the concentration of free Al^{III} in plasma is very low (< 5 μmol/L), computer simulations provide the only way of investigating aluminium speciation with respect to its bio-availability. For the purpose of computer simulations, trusty values of stability constants of low molecular weight (LMW) aluminium species, including these with amino acids, are needed.

The mechanism by which Al is absorbed, transported and excreted *in vivo* is largely, unclear. Probably, Al-induced alteration of cell membrane permeability may lead to facilitated transport of LMW or high molecular weight (HMW) Al-complexes with suitable ligands, across the membranes. Toxic action of Al could be direct – on nucleus chromatin or

indirect - by replacement of other elements and by inhibition of enzyme activity, such as lactate dehydrogenase, alkali phosphatase, carbonate anhydrase, catalase, etc. Al also may interfere directly with normal hemoglobin synthesis and porphyrin metabolism. Presence of Al^{III} in blood depresses retention of calcium, reduces absorption of phosphorus thus leading to lowering the ATP level and consequent disturbance of phosphorylation. Simultaneously the level of Al in bones, liver, brain and parathyroid gland increases. PTH hormone increases the absorption of Al in the gastrointestinal tract with resultant depression of iron absorption [5-7]. In tissues, the Al^{III} ion forms complexes with LMW ligands and according to the properties of these complexes (passive diffusion or dissolution across or into mucus matrices and lipid membranes) may be excreted or deposited [7]. In cells or in the blood stream, the aluminium ion may be complexed with the existing pool of amino acids. The complexes formed may either ameliorate aluminium absorption or act as carriers in the process of aluminium transportation to tissues [8 – 10]. It may be expected that the higher the stability constants are, the more efficient will be the competition for Al and the formation of Al species that can be taken up by the tissues. Berthon and Dayde [8] showed that dietary acids (malic, oxalic, tartaric, succinic, aspartic and glutamic) may dissolve a significant fraction of $Al(OH)_3$, from antacid formulations, and thus, form absorbable neutral Al-complexes which could cross the gastrointestinal membrane. The ameliorating effect of organic acids in gastrointestinal absorption of aluminium is most pronounced by citric and tartaric acid, though glutamic acid (from dietary wheat or corn) may play a significant role [9]. Matsumoto and Yamaya [10] found that malic, glutamic and citric acid reduced the Al-induced inhibition of a K^+-stimulated, Mg^{2+}- dependent plasma membrane ATP-ase in *Pisum sativum*. The inhibitory effect of Al is due to the formation of an Al-ATP complex and the amelioration by organic acids is due to the formation of stable complexes with Al.

In absorption, excretion and transport processes of aluminium, glutamic acid and serine (or phosphoserine) may have an important effect. Deloncle et al. [11] have shown that after chronic intoxication of experimental rats with i.v. injection of a suspension of sodium-L-glutamate and aluminum-chloride a significant increase of Al content occurred in different areas of the brain (hippocampus, occipito-parietal, cortex, cerebellum and striatum). These results were explained in terms of induced modification of the blood-brain barrier by Al-L-glutamate complex. Deloncle et al. [12] have also shown that Al is able to cross the erythrocyte membrane as a glutamate complex, in their study with in vitro distribution of Al, in the presence of Na-L-glutamate, between plasma and erythrocyte. CD spectroscopic studies [13] on two synthetic fragments of the human neurofilament protein midsized subunit (NF-M) and their serine substituted derivatives showed the formation of stable complexes of Al^{3+} with peptide ligands. In the case of Ser-phosphorylated fragments, Fourier transform i.r. spectra showed marked changes in the presence of Al. The OH group of serine facilitates the formation of Al-peptide complexes with the involvement of all carboxylate groups in the molecule. This result accentuates the importance of serine in modeling Al-protein interactions. It has been found that serine readily forms hydrogen bonded associates with $Al(OH)_3$ and so may have a solubilizing effect [14]. In this way modeling studies of Al-protein interactions require the knowledge of the stability constants of Al-amino acid chelates [15,16].

Despite this great potential biological interest literature data concerning identity and stability of Al-glutamate or Al-serinate complexes are still lacking. Since the aluminium(III) ion is very prone to hydrolysis [17] these studies are complicated by the formation of various hydrolytic and mixed hydrolytic complexes, mainly polynuclear, many of which are formed very slowly and are stable only over a very narrow range of pH's. This might be the reason why so far there exist only a few reliable literature data [18-23] concerning the identity and stability of the species formed in Al^{III}-amino acid systems. Duc et al. [18] using a potentiometric technique, found that Ai^{III} and L-glutamic acid, in 0.5 mol/L $NaClO_4$ medium at 298 K form the complexes Al(OH)HGlu and Al(HGlu) with the log stability constant 8.36 + 0.2, and 11.81 ± 0.3, respectively. Singh and Srivastava [19] found, using the same technique, that in 0.1 mol/L $NaClO_4$ medium at 298 K the aluminum(III) ion forms *mono*, *bis* and *tris* complexes with L-glutamic acid with a log consecutive stability constants, 15.12, 14.28 and 9.20, respectively. Yadava et al., [21] characterized the formation of *mono, bis and tris* serinato-aluminum(III) complexes by ionophoresis. Dayde [22] has characterized the formation of Al(HGlu), Al(Glu), $Al(H_2Glu)$, $Al_2H_{-1}Glu$ and $Al_2H_{-4}Glu$ complexes in the Al-L-glutamic acid system and the Al(Ser) complex in the Al-L-serine system at 37^0C in 0.15 mol/dm^3 NaCl medium. No other complexes with serine were found. Kiss et al. [23] have

recently reported some results on the complexation of the Al^{III} ion with a number of amino acids (Gly, Ser, Thr, Gin, Asn, Glu and Asp) as well as carboxylic (succinic) and substituted carboxylic acids (N-acetylaspartic and 2-sulfanylsuccinic). The speciation in the systems studied was derived from *p*H potentiometric, ^{1}H, ^{13}C and ^{27}Al NMR measurements. Potentiometric measurements were carried out in 0.2 mol/dm^3 KCl ionic medium at 25^{0}C. High concentration ratios of amino acid to aluminum were used with total concentrations of the amino acid up to 40 mmol/dm^3. With glutamic acid the species Al(HGlu), Al(Glu), Al_2Glu, and $AlH_{-1}Glu$ were identified while with serine Al(Ser), $AlH_{-1}(Ser)$ and $Al_2H_{-1}(Ser)$ complexes were found. Thus, both stoichiometries and stability constants of Al-Glu and Al-Ser complexes greatly vary depending on the different literature sources. Hence, the present study was aimed at characterizing the complexing process and establishing the conditions and possibilities for the existence of the particular complexes in the aluminum(III)-L-glutamic acid and aluminum(III)-L-serine systems, with the emphasis on ternary species. Experiments were carried out as potentiometric titrations using a glass electrode. Hydrolysis of the aluminum(III) ion and protonation of glutamate and serinate ions were studied in separate experiments. The pH range in complexation studies was restricted in such a way that the formation of pure hydrolytic complexes of aluminum(III) as well as the hydrolytic precipitation could be minimized.

EXPERIMENTAL

Reagents and Analysis

The stock solution of aluminum(III) chloride was prepared by dissolving doubly recrystallized $AlCl_3 . 6 H_2O$ p.a. (Merck) in twice distilled water. The appropriate amount of HCl was added to avoid initial hydrolysis of Al^{III}. The aluminum content was determined gravimetrically by the precipitation with 8-hydroxyquinoline and ammonia. Both methods gave the same results within 0.3%. The concentration of the free acid was determined potentiometrically using the Gran plot. The constancy of the total proton concentration with time was considered as a criterion for the absence of initial aluminum(III) hydrolysis and was periodically checked by titration against standard NaOH before each series of measurements.

L-glutamic acid p.a (Sigma) and L-serine p.a (Sigma) were dissolved in doubly distilled water and standardized by titration against standard NaOH.

The sodium hydroxide solution was prepared from a concentrated volumetric solution p.a. (Merck) by diluting with freshly boiled doubly distilled water, cooled under constant flow of purified nitrogen. The alkali concentration was checked by titration against potassium hydrogen phthalate. The hydrochloric acid solution was made from HCl "Suprapure" (Merck) and standardized against tris(hydroxymethyl) aminomethane. The solution of lithium chloride was prepared form LiCl, p.a (Merck) by dissolving the recrystallized salt in twice deionized water. The concentration was determined by evaporation of a known volume of solution to dryness at 573 K and weighing the residue.

Equipment

Potentiometric measurements were carried out using a Tacussel Isis 20000 digital pH-meter with a precision ± 0.1 mV (in some measurements an extended scale was used with a precision ± 0.01 mV). The pH meter was equipped with a Tacussel TC-100 combined electrode. Titrant was delivered from a Metrohm Dosimat model 665. A constant temperature was maintained with a VEB Prufgerate model E3E circulating ultrathermostat.

Procedure

All titrations were performed in a double mantled, thermostated glass vessel closed with aTeflon cork. The constant temperature (298.0 + 0.1 K) was maintained by circulating the thermostated water through the jacket. Purified and oxygen free nitrogen gas was bubbled through the solution for providing an inert atmosphere and stirring. Additional stirring of the solution was achieved with a magnetic stirrer.

The electrochemical cell used for the potentiometric measurements may be represented as: *RE*/test solution (*TS*)/*GE* where *RE* and *GE* denote reference and glass electrode respectively. The general composition of the test solution was: (a) in aluminum(III) hydrolysis studies: *TS* = *M* Al^{III}, *H* H^+, 0.1 mol/dm^3 Cl^- (b) in amino acid - Al^{III} complex formation titrations: *TS* = *M* Al^{III}, *H* H^+, *L* aa (aa = Glu^{2-}, or Ser^-), 0.1 mol/dm^3 Cl^-, where *M*, *H* and *L* denote total molar concentrations of the corresponding species.

The potential of the glass electrode is given by the expression:

$$E = E_0 + Q \log h + E_j$$

where h is the concentration of free protons, E_0 is a constant which includes the standard potential of the glass electrode, Q is the slope of the glass electrode response and E_j is a liquid junction potential whose contribution to E was found to be negligible. The E_0 was determined both before and during each titration of the test solution. First, E_0 was determined by means of a separate titration of HCl with sodium hydroxide, both of known concentrations, under the same medium and temperature conditions as the test solution titrations (1.0, 2.5 and 5.0 mmol/dm^3 HCl was titrated with 0.100 mol/dm^3 NaOH). The data so obtained were analyzed with the aid of the Magec [24] program. The calculated values were Q = 59.0 mV and the auto-protolysis constant of water, pK_w = 13.75(2). During the titrations of the test solutions, the E_0 was determined using the data in acidic medium, where no hydrolysis or complexation takes place ($h = H$), by plotting the value $E - Q \log h$ against h and extrapolating the straight line so obtained to $h = 0$. When the difference between two E_0 values was higher than 2.0 mV, the titration was rejected. Thus, the obtained value of E_0 was used for the calculation of $-\log h$ for the whole titration curve.

To reduce the concentration of the hydrogen ion, the titrant was added stepwise from an autoburette in small aliquots (0.005 - 0.01 mL). In pure hydrolysis studies a standard NaOH solution prepared in a 0.1 mol/dm^3 LiCl medium was used as titrant while in complexation studies besides standard NaOH the corresponding sodium salts of the amino acids *i.e.* sodium glutamate and sodium serinate were used as titrants. The salts were prepared by neutralizing the standard solution of the amino acid with an equivalent amount of standard NaOH. In this way some titrations were carried out according to Froneaus [25]. The titrants were added slowly, under energic stirring of the titrated solution. In this way initial formation of insoluble or colloidal aluminium hydroxide, which subsequently dissolves very slowly, was avoided. The presence of colloidal aluminium hydroxide, at *p*H values around 5, was difficult to observe. The indication that the titrated solution did not become supersaturated with respect to $Al(OH)_3$ was stable potential readings over prolonged periods of time (we arbitrarily chosen to monitor the potential, at the end of titration, for additional 3-4 hr.). During the titration the potential was monitored after each addition of a titrant. The readings were taken every 5 min until steady values to ± 0.1 mV/min were obtained. Hence, the average equilibration time (when hydrolysis occurred) for each point in hydrolysis measurements was 20 - 35 min while in complexation measurements it was 20 - 30 min, so that each titration lasted approximately two days. The titrations were terminated when drifted potential readings were obtained and turbidity of solutions observed. Some titrations were carried in duplicate and some in triplicate. Agreement between duplicate titrations better than 5%, constancy of the measured potential (to (0.1 mV or (0.002 *p*H uints) over prolonged period of time as well as constancy of E_0, served as criteria showing how close to thermodynamic equilibrium the reaction under investigation was.

Data Treatment

Three kinds of equilibria should be considered in the present study: (a) protonation of glutamate and serinate ions, (b) hydrolysis of aluminum(III) ion, and (c) general three component equilibria,

$$p\,Al^{3+} + q\,H^{+} + r\,aa \Leftrightarrow [Al_pH_q(aa)_r]^{(3p+q-r)+};\ \beta_{p,q,r}$$

which include the case q = 0, i.e. the formation of pure amino acidato complexes of Al^{III}. Negative values of q represent hydroxo complexes. The overall protonation constants of amino acidato ions and stability constants of hydrolytic complexes of aluminum(III) ions were determined in separate experiments. Thus, in the evaluation of three component equilibria (c), the binary models (a) and (b) were considered as known.

The mathematical analysis of the experimental data was performed with the aid of general least-squares program Superquad [26]. In Superquad calculations the identity and stability of complexes which give the best fit to the experimental data, were determined by minimizing the error-squares sum of the potentials, U:

$$U = \sum w_i (E_{obs} - E_{calc})^2$$

where w_i represents a statistical weight assigned to each point of titration curve, E_{obs} and E_{calc} refer to the measured potential of the cell and the calculated one assuming the specific model and trial constants, respectively. The best model was chosen using these criteria: (a)

the lowest value of U, (b) standard deviation in calculated stability constants less than 0.15 log units, (c) standard deviations in potential residuals, defined as:
$s = \{ewe^T / (N-k)\}^{1/2}$
where e is a vector in potential residuals (E_{obs} - E_{calc}), w is a weighting matrix, N is the number of observations and k is the number of refinable parameters, with standard deviation in volume readings 0.0005 cm^3 and standard deviation in potential readings 0.1 mV, should be less than 3.0. (d) goodness-of-fit statistics, χ^2 (Pearson's test) at 95% confidence level, with 6 degrees of freedom, less than 12.6 and (e) reasonably random scatter of potential residuals without any significant systematic trend. Along with Superquad the program Best [27] was also used in calculations. All calculations were performed on a PC IBM 586/200 compatible computer.

RESULTS AND DISCUSSION

In order to study the speciation and equilibria in the three-component system Al^{III} - aa - OH-(or H^+) where aa denotes L-glutamic acid or L-serine, it was necessary to evaluate the equilibria in the two-component system, i.e., to determine the protonation constants of glutamate and serinate ions as well as the stability constants of the hydrolytic complexes of Al^{III} under the same experimental conditions as for the complexation investigation. The need for studying hydrolysis is potentiated by the fact that the speciation model, in case of weak complex formation, substantially depends upon the hydrolytic model used in the calculations.

Protonation Constants of L-glutamate and L-serinate ions

Since we could not find the relevant protonation constants of glutamate and serinate ions, related to our experimental conditions, in the available literature [28], these were determined in 0.1 mol/dm^3 LiCl ionic medium at 298 K. Four titrations with total concentrations of L-glutamate 5.0, 2.5, 1.2 and 0.6 mmol/dm^3 in the pH range 1.820 - 11.450 were carried out. Three titrations with total concentrations of L-serinate 10.0, 5.0 and 2.5 mmol/dm^3 in the pH range 1.980 - 11.800 were performed. Approximately 150 potential - volume readings were collected per titration. The data were analyzed using the Magec and Superquad programs. The calculated overall protonation constants were found to be for L-glutamate: log $ß_1$ = 9.57 ± 0.01; log $ß_2$ =13.93 ± 0.02; log $ß_3$ = 16.08 ± 0.04 and for L-serinate: log $ß_1$ = 8.97 ± 0.02; log $ß_2$ = 11.25 ± 0.05. These results are in good agreement with literature data under similar medium and temperature conditions [28].

Hydrolysis of Aluminum(III)

Although the hydrolysis of aluminum has been the subject of extensive studies over the past few decades there still exists some controversy regarding the identity and stability of the species formed in solution. Various factors, such as: the method of preparation of the solution, the degree of hydrolysis (defined as the molar ratio m = [OH]/[Al]), pH range, temperature, concentration of reactants, nature and concentration of supporting ionic medium, type and the rate of the base addition), have a great influence on the composition and stability of the hydrolytic complexes formed in solution of the Al^{III} ion. Therefore, a wide variety of hydrolytic aluminum species have been proposed to exist in solution by different authors. This subject has been reviewed by Baes and Mesmer [17], Akitt [29], Orvig [30], Bertsch [31], Martin [32] and others. The common consensus is that at lower values of m < 1 and low concentrations of aluminum (< 5 mmol/dm^3) in the pH range 3.5 - 4.5, the main hydrolytic products of aluminum are $Al(OH)^{2+}$, $Al(OH)_2^+$, and $Al_3(OH)_4^{5+}$. At very low aluminum concentrations (μmol) Martin assumed the formation of mononuclear hydrolytic complexes only, $Al(OH)_n$ (n = 1-3). At pH > 6 the complex $Al(OH)_4^-$ is a major species. Concerning the existence of the dimer $Al_2(OH)_2^{4+}$ there has been a disagreement. Some authors [33] included the dimer in the speciation scheme while others could not confirm its existence [34]. At higher aluminum concentrations and m ≤ 2 the $Al(OH)^{2+}$ and the tridecamer, $AlO_4Al_{12}(OH)_{24}(OH_2)_{12}^{7+}$ were found to be the principal constituents of the hydrolyzed solutions along with some transient oligomers with Al to OH molar ratio between 1:1.5 and 1:3.5. No data of aluminum hydrolysis in LiCl medium could be found in the literature. Hence, in view of afore presented results (especially concerning the existence of the dimer, $Al_2(OH)_2^{4+}$) it has been necessary to determine the composition and stability of the main aluminum hydrolytic species, under the same experimental conditions as for the aluminum-amino acids complexation study.

The experimental data obtained in the 0.1 mol/dm^3 LiCl medium, at 298 K are summarized in Table 1. In the medium used the aluminum(III) hydrolyzes between pH 3.5 and 4.5 dependent on its concentration.

Table 1. Summary of potentiometric data on aluminum(III) hydrolysis in 0.1 mol/dm^3 LiCl ionic medium, at 298 K. Concentrations C(X), of the corresponding species are given in mmol/dm^3. Z_{max} is the maximum value of the average hydrolytic number attained; No is the number of points included in calculations.

Entry	C(Al^{3+})	C(HCl)	pH range	Z'_{max}	No
1	4.970	3.600	2.393 - 4.185	0.312	25
2	2.490	3.320	2.436 - 4.285	0.273	21
3	0.990	3.086	2.494 - 4.417	0.313	20
4	0.600	1.150	2.952 - 4.710	0.430	30

The maximum values of average hydroxide number, Z, defined as $Z = (h - H)/M$, were ca. 0.43. The data shows that for each concentration of the aluminum studied, a separate titration curve, $Z = Z(- \log h)$ was obtained. This indicates the formation of polynuclear hydrolytic complexes. The *p*H region in which hydrolysis occurs depends upon the total concentration of aluminum. Thus, as the concentration of the aluminum(III) ion increases, the beginning of hydrolysis shifts towards lower *p*H values, while at the same time the degree of hydrolysis decreases. Possible complexation of aluminum(III) ion with chloride from the ionic medium, should appear as a constant effect because of relatively high concentration of the medium; therefore, it should not affect the number of hydroxide ions bound to aluminum. Though the titrations were performed in a wide *p*H range, for the purpose of calculations some reduction of the number of titration points was necessary. Points at low *p*H values, where hydrolysis is negligible and at *p*H's higher than 4.6, where solutions became turbid and colloid formation may take place, were excluded from calculations.

Reacting with water molecules, the aluminum(III) ion forms one or more hydrolytic complexes of the general composition $Al_p(OH)_q^{(3p - q)+}$ (further abbreviated as (p,q)) whose overall formation constants, $ß_{p\,q}$ can be defined as:

$$\beta = C_{p,q} m^{-p} h^{q}$$

where $C_{p,q}$ denotes the equilibrium concentration of the (p,q) complex and m is the free concentration of Al^{III} ion. In the above reaction, the chloride ions and water molecules are omitted from the coordination sphere.

First, each titration curve was processed separately using the program Best. On the basis of hydrolytic curves $Z = Z(- \log h)$, the *p*H interval chosen was 3.0 – 4.4. Complexes from the initial set comprising (1,-1), (1,-2), (1,-3), (1,-4), (2,-1), (2,-2), (2,-3), (2,-4), (2,-6), (3,-3,), (3,-4), (3,-6), (6,-12), (6,-15), (6,-18), (8,-12) and (13, -32) were introduced one at a time until the minimum value of s_{fit} [27] was obtained. During the calculations all the titration parameters (M_0, H_0) were kept constant while *p*H values in repeated cycles of calculations were adjusted until the best possible value of s_{fit} was obtained. The accepted set of complexes was (1,-1), (1,-2), (2,-2), (2,-4) and (3,-4). Then all titration curves were processed together, this time using the program Superquad. In Superquad calculations the E_0 values were allowed to float while all analytical parameters were held constant. All the complexes found in Best calculations were accepted with small difference in stability constants. Stepwise introduction of other complexes from the initial set, lead either in their rejection or in much worse set of statistical parameters determining goodness of fit. No higher polymers were accepted neither was the

tridecamer. Since, however, residuals showed a systematic trend at the beginning and at the end of *p*H interval chosen, the number of points used in the calculation was reduced, so that only the points from *p*H 3.7 to 4.6 were included in calculations. Repeated calculations ended with the rejection of the (2,-4) complex and acceptance of tridecamer, $Al_{13}(OH)_{34}^{7+}$. At the same time the stability constant of the dimer (2,-2) lowered with a higher standard deviation. Other complexes remained practically unchanged. Generally, the fit was improved in terms of better scatter of residuals and lower value of s. The next iteration was forced with manual exclusion of the (2,-2) complex from the model. No significant changes in the model were observed, except a slight rise of χ^2 (from 12.1 to 12.9). Thus, the dimer (2,-2) was, with due precautions, accepted in the hydrolysis speciation model, but in complexation calculations it was included as a refinable species. The final results of calculations are given in Table 2 together with the calculated statistical parameters.

Table 2. Composition and formation constants of the hydrolytic and the species in the L-Glu (and L-Ser) + Al^{III} systems. Values for the statistical parameters of the fit are given.

Species	log ($\beta_{p,q,r} \pm \sigma$)	
	Superquad [26]	Best [27]
$Al(OH)^{2+}$	- 5.62 ± 0.04	-5.67 ± 0.06
$Al(OH)_2^{+}$	-9.76 ± 0.01	- 9.78 ± 0.02
$Al_2(OH)_2^{4+}$	- 7.36 ± 0.08	- 7.00 ± 0.02
$Al_3(OH)_4^{5+}$	- 13.73 + 0.06	- 13.60 ± 0.12
$AlO_4Al_{12}(OH)_{24}^{7+}$	- 106.2 ± 0.1	- 109.6 + 0.12
Statistical parameters	χ^2= 11.5-12.9, s= 2.0-3.0	σ_{fit} = 0.002-0.010
$Al(HGlu)^{2+}$	12.02 ± 0.04	11.76 ± 0.05
$Al(Glu)^{+}$	7.86 ± 0.01	7.64 ± 0.02
$AlH_{-2}Glu^{-}$	- 2.30 ± 0.08	-
$AlH_{-3}Glu^{2-}$	- 8.44 ± 0.10	- 8.07 ± 0.01
Statistical parameters	χ^2=12.6-12.9, s = 1.05-2.9	σ_{fit} = 0.002 – 0.011
$Al(HSer)^{3+}$	11.18 ± 0.10	11.03 + 0.05
$Al(Ser)^{2+}$	5.71 ± 0.02	5.52 ± 0.03
$Al_2H_{-1}Ser^{4+}$	4.65 ± 0.03	4.10 ± 0.12
$AlH_{-2}Ser$	- 2.51 ± 0.04	- 2.40 ± 0.01
$AlH_{-3}Ser^{-}$	- 7.4 ± 0.1	-
Statistical parameters	χ^2=11.4 – 12.6, s = 2.1 – 2.8	σ_{fit} = 0.003

The set of statistics obtained as well as scatter of residuals indicate a good fit of the data. The distribution of the species over a *p*H range between 3.0 and 5.0 using the program Species [35] indicates that hydrolytic complexes occur, in significant concentrations, between *p*H 3.9 and 4.5. Low molecular weight complexes are the principal constituents of the hydrolyzed solution of Al^{III} ion bellow *p*H 4.5 while, beyond this value, the concentration of tridecamer sharply increases: it is probably the precursor of the polymers which eventually precipitate from the solution. Formation of either soluble or amorphous $Al(OH)_3$ was not detected under our experimental conditions.

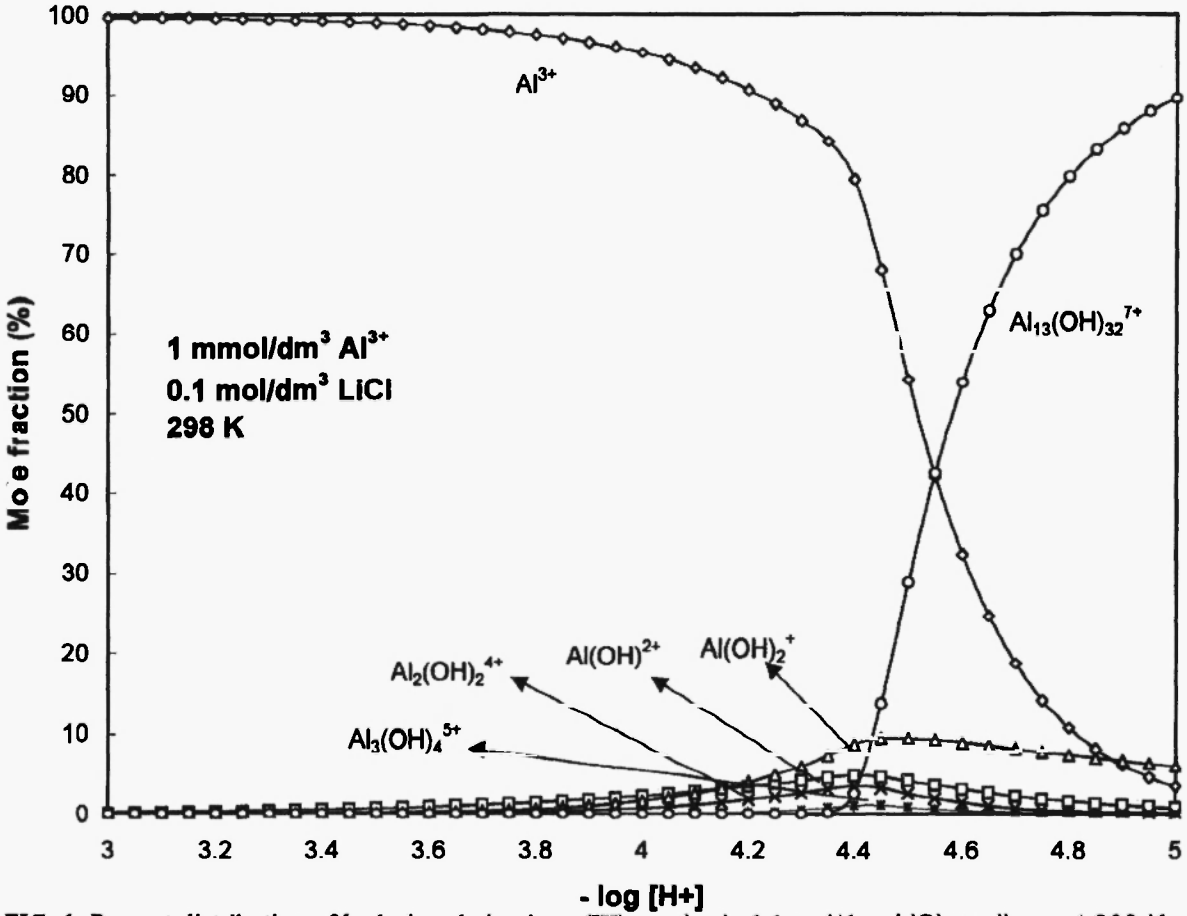

FIG. 1. Percent distribution of hydrolyzed aluminum(III) species in 0.1 mol/dm3 LiCl medium, at 298 K. Total concentration of Al^{III} is 1 mmol/dm³.

The Aluminum(III) - L-glutamic acid system

The experimental data obtained by emf measurements in 0.1 mol/dm³ LiCl medium at 298 K are summarized in Table 3. In the *p*H range studied (2.4 - 5.6) the maximum apparent ligand number reached was ca. 1.5. The highest concentration ratio of Glu to Al^{III} was 5:1. Beyond the *p*H 6.0, solutions became turbid and drifting potential readings were obtained. No higher concentration ratios of Glu to Al were used because they would seriously change constancy of the medium and in addition, the strong buffering effect of glutamic acid will hinder the reliable potentiometric measurements. The obtained formation curves, as the dependence of average ligand number, Z_c on - *log* [Glu], indicate extensive hydrolysis in the system and the formation of mononuclear complexes. The average ligand number was calculated with the relation:

$$Z_c = \frac{C_{Glu} - \frac{\{H - h + [OH]\}}{Z_H}}{M}$$

where Z_H denotes the average proton number of glutamate ion. From the maximal Z_c values attained, it can be seen that mixed hydrolytic complexes may be important.

Table 3. Summary of potentiometric titrations of L-glutamic acid + Al^{III} in 0.1 mol/dm^3 LiCl medium at 298 K. All concentrations are in mmol/dm^3. L is the total initial concentration of L-Glu and M that of Al^{III}. Z_{max} is the maximum value of average ligand number attained.

Entry	C(Al^{3+})	C(HCl)	C(Glu^{2-})	L/M	pH range	Z_{max}
1	2.49	2.22	12.5	5.02	2.677 - 5.140	2.178
2	0.99	2.08	5.00	5.05	2.962 - 5.229	1.985
3	4.97	2.45	15.02	3.02	2.983- 5.650	1.832
4	2.49	2.22	7.50	3.01	3.018 - 5.237	2.026
5	0.99	2.08	3.04	3.07	2.922 - 5.022	1.494
6	4.97	2.45	7.51	1.51	2.871 - 5.183	0.817
7	2.49	2.22	3.72	1.49	2.908 - 5.208	0.887
8	0.99	4.00	1.49	1.51	2.415 - 4.960	1.364

The equilibria in Glu + Al^{III} system may be represented in a general form:

$$p\,Al^{3+} + q\,H^{+} + r\,Glu^{2-} \rightleftharpoons Al_pH_q(Glu)_r$$

The stability constants of various (p,q,r) species formed in the above reaction, may be defined as:

$$\beta_{p,q,r} = C_{p,q,r} m^{-p} h^{-q} a^{r}$$

where $C_{p,q,r}$ denotes the equilibrium concentration of the complex, m, h and a denote free concentrations of aluminum(III), proton and glutamate, respectively. Negative values of q represent hydroxo complexes. To determine the composition and stability constants of the species formed the titration data were analyzed using the programs Best and Superquad. The following complexes were selected to find the model which best fit the experimental data: (1,0,1), (1,0,2), (1,0,3), (1,1,1), (1,2,1), (1,1,2), (1,2,2), (1,-1,1), (1,-2,1), (1,-3,1), (1,-1,2), (1,-2,2), (1,-1,3), (1,-2,3) and polymers (2,1,1), (2,2,1), (2,1,2), (2,2,2), (2,-1,1), (2,-2,1), (2,-2,2), (2,-3,1), (2,-3,2), (3,-1,1), (3,-2,1), (3,-1,2), (3,-2,2). More than 20 various models were tested. During the calculations analytical parameters (M_0, H_0 and L_0) were held constant while E_0 values were allowed to float. The hydrolytic complexes and protonated species of glutamate were not refined during the calculations except the hydrolytic dimer (2,-2). First, each titration curve was treated separately using the program Best. Complexes were added in the model one at a time until the lowest value of s_{fit} was achieved (usually less than 0.003). These complexes were used as the starting model for the Superquad calculations. The following complexes were included: (1,0,1), (1,1,1), (1,-1,1), (1,-2,1), (1,-3,1) and (2,-2,2).Then the data belonging to all titration curves referred to one particular glutamate to aluminum concentration ratio, were treated together. The refined values of E_0 served as the additional criterion for model selection. If they were different from the experimental ones by more than 0.5 mV the model was considered as inadequate. The results of calculations indicate that no polynuclear complex formation takes place. Also, the *bis*-complex, $Al(Glu)_2$, was not accepted in any model tested. The hydrolytic dimer (2,-2) was rejected in a first iteration cycle. In order to check the reliability of the model finally accepted all the stability constants and E_0 values were held constant while H_0 values were optimized.

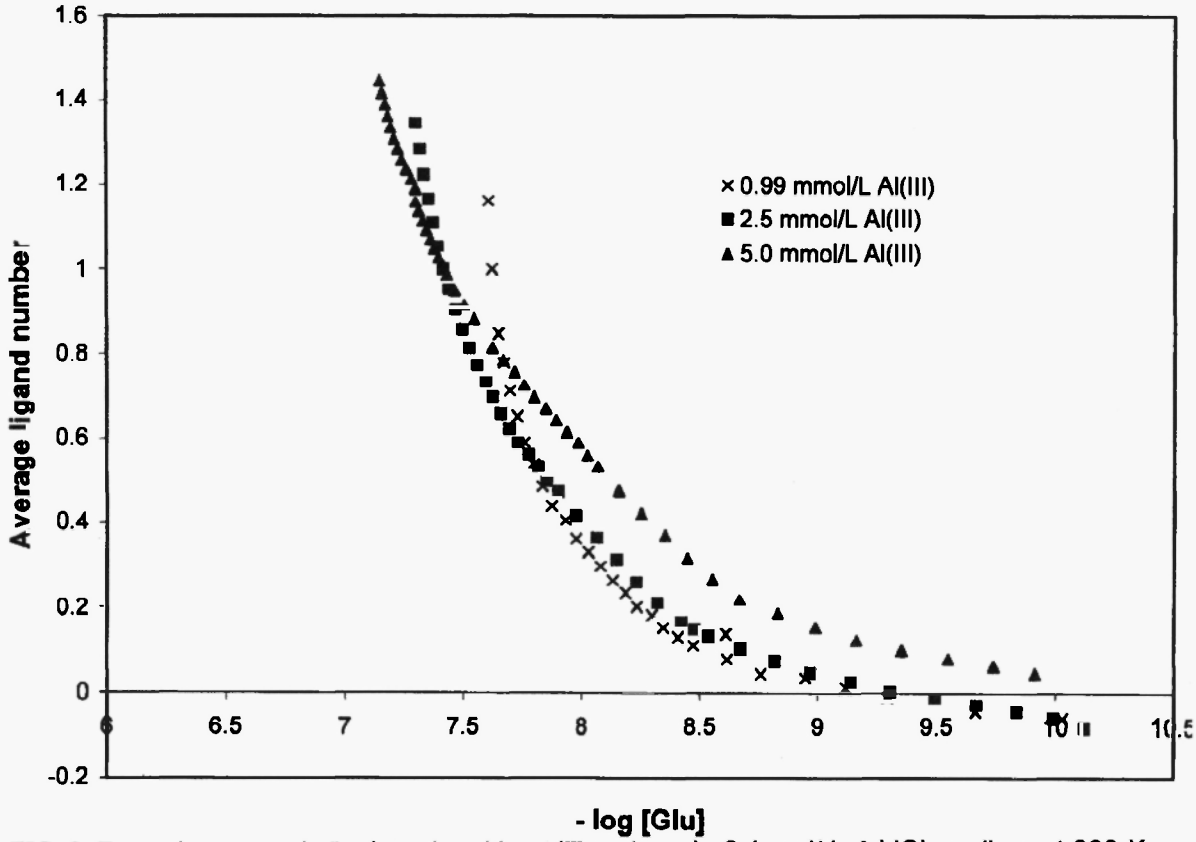

FIG. 2. Formation curves in L-glutamic acid + Al^{III} system, in 0.1 mol/dm³ LiCl medium at 298 K. Concentration ratio [Glu]: [Al] = 5: 1

The calculated total initial proton concentration did not deviate more than 1% from the experimental ones. This indicated the reasonably good fit of the data. The results of the calculation are presented in Table 2 together with the calculated set of statistical parameters. The distribution of various complexes in solution is shown in Fig. 3. The dominating complex at pH values lower than 4 is the protonated complex Al(HGlu). Upon increasing the pH this complex begins to protolyse giving the Al(Glu) complex. This binary complex reaches the maximum concentration at *p*H 4.5. At pH values higher than 4.8 the concentration of mixed hydrolytic complexes becomes significant. They may act as precursors of the species which eventually precipitate from the solution. Concentration of low molecular weight pure hydrolytic complexes is less than 5%. The concentration of the tridecamer sharply increases from *p*H 4.5 to 5.5 and then begins to fall. Near *p*H 6.0 the only important complexes are the mixed hydrolytic monomers $AlH_{-2}Glu^{-}$ and $AlH_{-3}Glu^{2-}$. Being negatively charged these complexes may utilize the anionic channels in the cell membrane to enter the cell. However, crossing of both amino acids and their complexes with metal ions, through a cell membrane is a complicated process which depends upon not only the charge of the complex, but also on strength of the bond between amino acid and metal ion as well as the shape of the molecule. It also depends on the cell type. It is therefore difficult to discuss the effect of amino acids on cellular uptake of aluminum and its transfer through the cell membrane on the basis of very scarce experimental data. The obtained stability constants of glutamato-aluminum(III) complexes indicate that glutamate assisted cellular uptake of aluminum should be much lower than that of carboxylic acids (citric and tartaric). It would be of interest to make a comparison with glutaric and ketoglutaric acid but it seems that presently, there is no available experimental data.

In Al(HGlu) the amino group is not involved in the coordination since it is known that the affinity of aluminum to nitrogen donor is very low [36]. Thus, only the α-carboxyl group is coordinated to Al^{III} in this complex. Therefore, the actual composition of the Al(Glu) complex may be Al(OH)HGlu i.e. the $HGlu^{-}$ ion is attached to the monohydroxo complex, $Al(OH)^{2+}$. Similarly, the complexes $AlH_{-2}Glu$ and $AlH_{-3}Glu$ may be formulated as $Al(OH)_3HGlu$,

and $Al(OH)_4HGlu^{2-}$, rescpectively. The ability of glutamate to be attached to the hydroxo-aluminum(III) core may be of importance in its interaction with "$Al(OH)_3$" from antacid formulations.

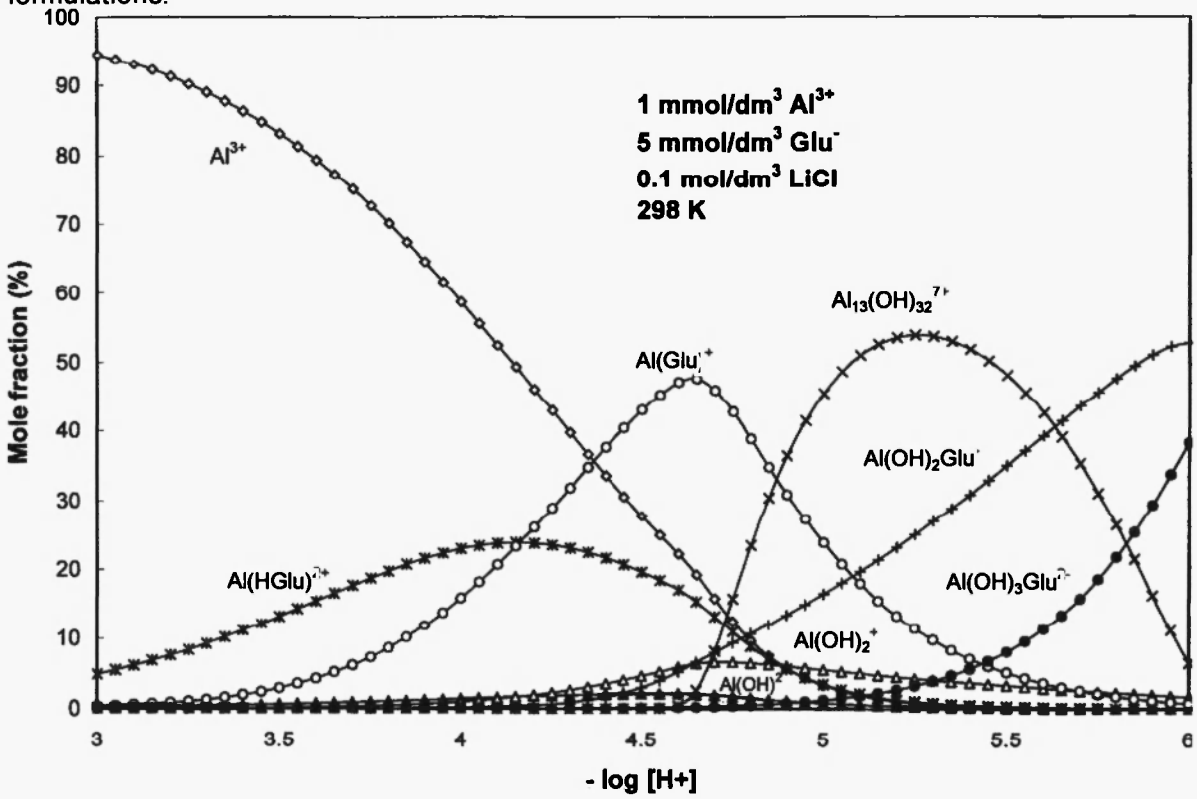

FIG. 3. Percent distribution of species formed in L-Glu + Al^{III} system. Total concentration of Al is 1 mnmol/dm³ with [Glu]/[Al] = 5:1.

The Aluminum(III)-L-Serine System

The experimental data concerning the glass electrode potentiometric measurements in 0.1 mol/dm³ LiCl ionic medium, at 298 K are given in Table 4. The equilibrium in this system was reached much slowly than in the Al^{III}-glutamic acid system. It was necessary to wait more than 2 hours to obtain stable potential readings at *p*H values higher than 3.0. Hence, a part of the titrations was carried out according to Froneaus [25], i.e. acidified solutions of Al^{III} in LiCl ionic medium were titrated with sodium-serinate solutions, prepared in the same ionic medium. In this way the equilibration time was reduced to half an hour per point. The maximal concentration ratio of serine to aluminum attained was 6:1. The obtained formation curves (Fig. 4.) indicate extensive hydrolysis and polynuclear complex formation.

The obtained data were treated in the same manner as for the aluminum(III)-glutamic acid system. It appeared that no plausible set of complexes could fit experimental curves. Therefore, the calculation strategy described by Gillard et al.,[37] was applied. From the initial part of the titration curves the stability constant of $Al(HSer)^{3+}$ was calculated. Taking this constant as known, the Best and Superquad programs were used to calculate the stability constants of other species now including the full set of titration points. The results obtained, indicate that at the all concentration ratios of Ser to Al^{III} the mixed monomer $AlH_{-2}Ser$ is formed. Generally however, the fit was unacceptable with the scatter of residuals showing a systematic trend. To improve the fit, the stability constants of (1,1,1) and (1,-2,1) complexes were fixed while the stability constants of pure hydrolytic species were varied. The data were treated by introducing various new complexes in the model, one at a time.

Table 4. Summary of potentiometric titrations of L-serine + Al^{III} solutions in 0.1 mol/dm^3 LiCl medium, at 298 K. All concentrations are in mmol/dm^3. L is total initial concentration of serine and M that of aluminum. Z_{max} denotes maximum average ligand number attained. In the first calculation cycle the hydrolytic complex (2,-2) was rejected with significant improvement of the fit. In a second cycle no further improvement of the fit could have been achieved (lowering s or σ_{fit}) so that the stability constants of the remaining hydrolytic complexes were fixed and a new calculation cycle was initiated by a systematic introduction of mixed complexes.

Entry	C(Al^{3+})	C(HCl)	C(Ser^-)	L/M	pH range	Z_{max}
1	9.95	6.90	-	1.00	2.162-4.264	0.822
2	4.97	6.43	-	1.50	2.192-4.375	0.983
3	2.49	6.20	-	2.60	2.210-4.562	1.443
4	0.99	6.06	-	6.10	2.218-5.280	2.978
5	4.97	6.43	25.00	5.03	2.714-4.200	0.416
6	2.49	6.20	12.50	5.02	2.528-4.422	0.834
7	0.99	6.06	5.00	5.05	2.339-4.731	1.698

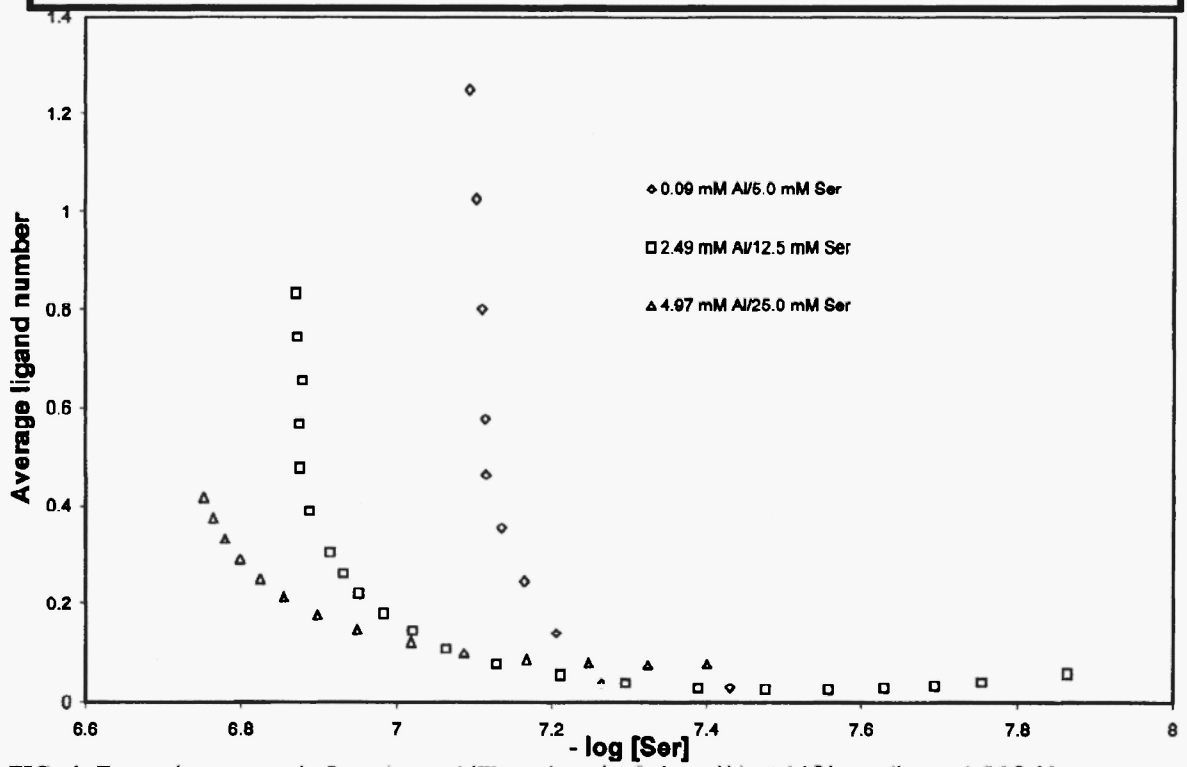

FIG. 4. Formation curves in L-serine + Al^{III} system in 0.1 mol/dm^3 LiCl medium at 298 K.

First, the complex Al(Ser) was tested and was accepted with slight improvement of the scatter of residuals. Since at higher *p*H values the scatter still was unacceptable, polynuclear complexes were introduced in the calculations. The expected complex (2,-2,2) was rejected, but the complex $Al_2H_{-1}Ser$ was accepted. Other polynuclears were not accepted. With the obtained set of complexes we calculated the titration curves and plotted calculated curves vs. experimental ones. Good agreement was obtained at *p*H values lower than 4.5. Agreement at higher pH values was poor. The acceptable fit ($s < 3.0$ in Superquad or $\sigma_{fit} < 0.05$ in Best calculations) was finally obtained upon introducing the complex (1,-3,1).

The complexes found, together with their respective stability constants, are given in Table 2. The distribution diagram of the various species formed in solution is shown in Fig 5. It can be seen that the mononuclear, protonated complex $Al(HSer)^{3+}$ is the dominating species over a broad pH range between 1.0 and 4.0. Bearing in mind the pH region, in which this complex is formed, it is reasonable to suppose that its formation proceeds according to the reaction:

$$Al^{3+} + HSer^{\pm} \rightleftharpoons Al(HSer)$$

i.e. the "zwitterion" $HSer^{\pm}$ reacts with the hydrated aluminum(III) ion and forms the complex. It may be supposed that aluminum is bound to serine through the carboxylate group. The amino group does not participate to the coordination. If we compare the stability constant of the Al(HSer) complex, log K, calculated from the afore stated equilibrium, with the one for lactic acid [28], log β:

$$\log K = \log \beta_{1\,1\,1} - \log \beta_{1\,1\,0} = 11.16 - 8.97 = 2.19$$

$$\log \beta = 2.36$$

we can see that the similarity of these values justifies the conclusion that in the Al(HSer) complex only the carboxylate group is bound to aluminum.

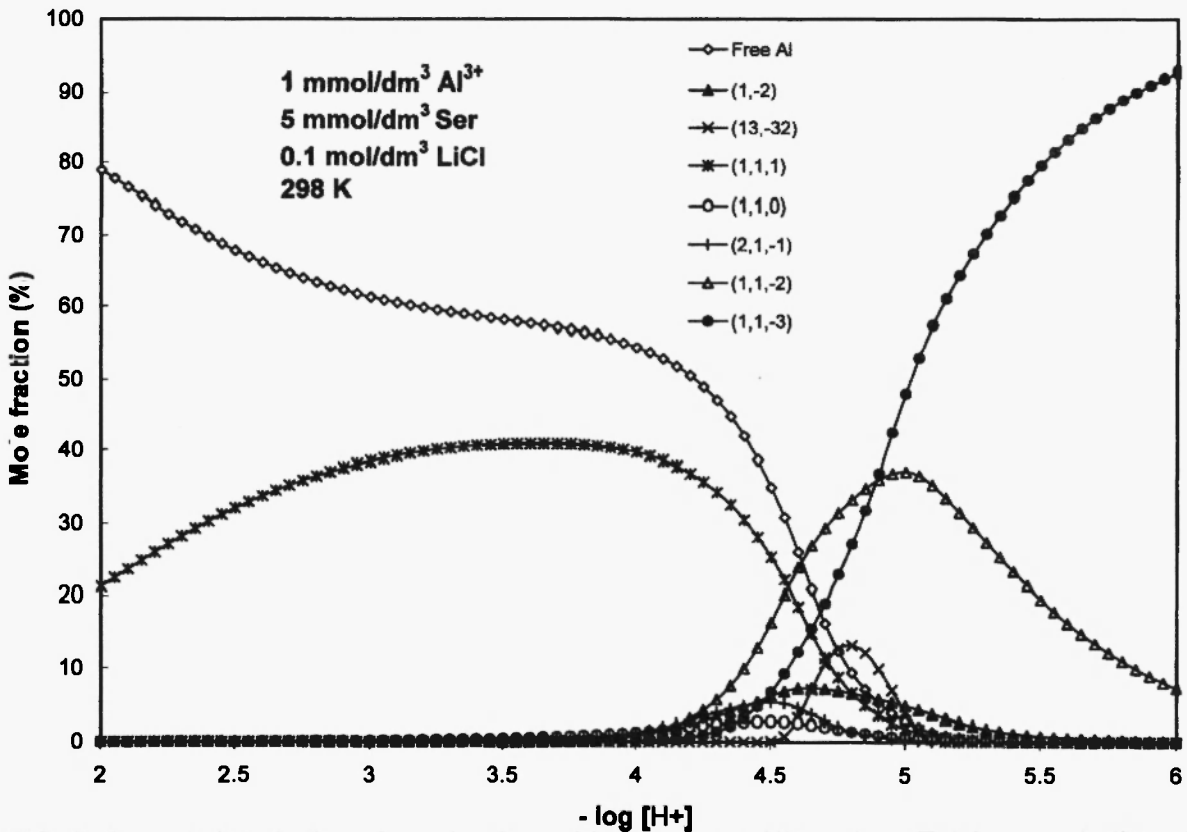

FIG. 5. Percent distrubution of species formed in L-serine + Al^{III} system. Total concentration of Al^{III} is 1 mmol/dm3 with [Ser]/EAl] = 5:1.

Upon increasing the *p*H this complex dissociates fast and reversibly, but the $Al(Ser)^{2+}$ which is formed, has a low concentration with a maximum at *p*H 4.5. The reason is probably its

extensive hydrolysis to mixed $Al(OH)_2$ and $Al(OH)_3$ complexes. Alternatively the mixed hydrolytic complexes could be formed by a direct reaction of the HSer± "zwitter" ion with $Al(OH)_3$ and $Al(OH)_4^-$ respectively. Such a mechanism was proposed earlier for the formation of the mixed acetato-aluminum(III) complexes in solution [38]. The dinuclear complex may be formed according to reaction:

$$Al(OH)_2^{2+} + Al(HSer) \rightleftharpoons Al_2(OH)Ser + H_2O$$

Since itsconcentration is low it seems that the hydrolysis of Al(Ser) is the dominating reaction in the *p*H interval 4.5 – 5.5.
In table 5 the results obtained in this work and available literature data are compared.

Table 5. Overall stability constants of species formed in L-glutamic acid + Al^{III} and L-serine + Al^{III} systems

Species	log $\beta_{pqr} \pm \sigma$			
	this work[a]	Ref. 23[b]	Ref. 22[c]	Ref. 18[d]
Al(HGlu)	12.02	10.88	11.07	11.81
Al(Glu)	7.86	7.29	7.69	8.36
Al_2(Glu)	-	9.46	-	-
AlH_2Glu	-	-	14.74	-
$Al_2H_{-1}Glu$	-	-	6.45	-
Al_2H_4Glu	-		- 6.71	
$AlH_{-1}Glu$	-	2.55	-	-
$AlH_{-2}Glu$	-2.30	-	-	-
$AlH_{-3}Glu$	-8.44	-	-	-
Al(Hser)	11.16	-		
Al(Ser)	5.71	5.66	5.97 ± 0.05	
$AlH_{-1}Ser$		0.62		
AlH_2Ser	- 2.51	-		
AlH_3Ser	-7.5	-		
$Al_2H_{-1}Ser$	4.65	3.75		

a) 0.1 M LiCl, 298 K; b) 0.2 M KCl, 298 K; c) 0.15 M NaCl, 310 K; d) 0.5 M $NaClO_4$, 298 K

One can conclude that differences between speciation schemes and the stability constants proposed, may be attributed primarily to the different hydrolytic models and experimental protocol employed.
The results of the present study indicate that in the title systems competition between many Al equilibria exists, so that the transport and accumulation of aluminium must be considered in

terms or these equilibria, taking place in cells or in blood. Kinetic factors play an equally significant role. The dominance of amino acids – aluminium ion equilibria may be expected in tissues and compartments where the concentration of free amino acids is high, as well as their concentration ratio to aluminium. Such a situation may arise in kidneys since the fraction of aluminium, which is not bound to serum transferrin, is excreted in kidneys [39]. Glutamate and serinate ions in ultrafiltrates may complex aluminum and, if charged, these complexes may enhance the excretion of Al. Normal plasma concentration of glutamic acid is 48, serine 107 and aluminium (at most) 5 μmol/L [40]. Similar concentration should be expected in ultrafiltrate except for aluminium which should be smaller. The binding capacity of glutamate and serinate for aluminium may be estimated on the basis of *p*Al values which, according to table 5, is 11.7 for glutamate and 13.2 for serinate (for total aluminium concentration 1 mmol/L and amino acids concentration 50 mmol/L, at *p*H = 7.0) [7]. These values are comparable with those for phosphate. Thus, bearing in mind slightly acidic *p*H in kidneys, one may expect the formation of mostly binary or mixed hydrolytic complexes between aluminium and glutamate or serinate. Since they are all charged, excretion may be expected to be enhanced. However, aluminium may me also deposited in kidneys, depending on exposure to exogenous Al-compounds, age, physiological state of organism, etc. Its toxic effect is mostly pronounced on proximal tubules. In pathological cases, when tubular reabsorption of amino acids is greatly reduced (so that they reach about 10 to 20 times higher concentration in primary urine than physiological), formation of neutral or mixed hydroxo aluminium complexes may prevail. Consequently, mobilization of aluminium from kidney deposits may occur, since according to distribution diagrams in Figs. 3 and 4 formation of mixed hydroxo amino acidato complexes of aluminium may prevent the formation of insoluble aluminium phosphates.

The formation of protonated aluminium complexes with "zwitter" ionic forms of amino acids may also be a factor that increases aluminium toxicity. If high concentrations of glutamate are ingested (e.g. in the form of food additives such as sodium hydrogenglutamate) concomitantly with aluminium based antacids then, in pathologically altered gi membranes, absorption of aluminium may occur with consequent toxic effects.

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